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Research Article





THE CONCENTRATION AND SIGNIFICANCE OF THE BUTANOL-EXTRACTABLE I¹⁸¹ OF SERUM IN PATIENTS WITH DIVERSE STATES OF THYROIDAL FUNCTION

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Thyroxine, if not truly the active thyroid hormone (1, 2) is clearly the principal iodine-containing compound released by the thyroid gland, and ordinarily comprises the largest fraction of the plasma's protein-bound iodine (3–6). Many investigators have, therefore, attempted to assess thyroidal function by measuring the concentration of protein-bound I¹⁸¹ (PBI¹⁸¹) in the plasma following the administration of radioactive iodine (7–11). The PBI¹⁸¹ (12), or even the total radioactivity in the plasma (13, 14), determined several days after the administration of the tracer, reflects the radioactivity of circulating thyroxine, except in patients who have received large doses of I¹⁸¹ (15).

It has been shown that the hormonal iodine which is extractable from serum with butanol and not re-extractable with alkali (hereafter referred to as the butanol-extractable iodine) is composed largely of thyroxine. Its concentration in serum differs significantly from that of the protein-bound iodine (16).

The present communication reports the results in 109 patients with varying states of thyroidal function in whom the plasma's concentration of radioactive butanol-extractable iodine was determined both one and three days after the administration of I¹⁸¹. From a correlation of these results with measurements of the amount of I¹⁸¹ accumulated by the thyroid gland, an estimate of both the amount and the rate of turnover of glandular hormone has been made.

MATERIALS AND METHODS

The present study includes all patients referred to the Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center during the period between January, 1952 and June, 1952 for the determination of 24-hour thyroidal uptake of radioactive iodine. Since no attempt was made to select cases within this group, which included dependents as well as military personnel, the population studied is probably representative of that referred to the thyroid clinic of any large hospital. Each patient was questioned and examined by one or another of the authors, and a diagnostic impression was recorded prior to the administration of I²²¹. This record, together with the result of the iodineuptake test, was reviewed by one of the authors (S. H. I.), and each patient was assigned a definite diagnosis before the results of the butanol-extraction procedure were known. Prolonged observation of the patients, in an attempt to ascertain the accuracy of the diagnosis, was usually impossible.

A standard amount (50 to 100 \(mu\)c) of I¹²¹, free of carrier, was administered orally to each patient. The patient returned 24 hours later for the determination of the thyroidal uptake of the tracer. This was carried out with a shielded Geiger-Mueller tube (bismuth-sputtered-cathode) placed 20 cm. from the neck. The radioactivity in the neck was compared with that of a 50 ml. volumetric flask, similarly positioned, containing an amount of I¹⁸¹ equal to the dose administered. In most patients the thyroidal content of I1st was also determined 48 and 72 hours after the administration of the tracer. The following convention was adopted as an index of the preponderance of either accumulation of radioiodine or the release of radioactive hormone during the interval between 24 and 72 hours after the administration of I's. When the thyroidal uptake displayed a progressive increase during this period, the 24-hour uptake was subtracted from the 72-hour uptake and the difference ("net change") was given a positive sign. When the uptake decreased at any time during the period, the lowest value was subtracted from the highest and the difference was given a negative sign.

During the 24 and 72-hour visits, blood was drawn for the measurement of the concentration of butanol-extractable I^{IM} (BEI^{IM}). Separation of the serum was carried out as soon as clot retraction had occurred. In virtually all cases, the analysis was begun on the same day the blood was drawn.

The method used in the extraction of thyroxine from serum was an adaptation of that described by Man, Kydd, and Peters (16). Five cc. of serum were brought to a pH of approximately 3.5 with 10 per cent H_2SO_4 , using

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a few drops of bromphenol-blue as an internal indicator. Twenty-five cc. of n-butyl alcohol were then added in a fine stream, while the serum was continuously agitated. The resulting mixture was centrifuged at 2500 r.p.m for 8 minutes. The supernatant butanol was transferred to a 125 ml. centrifuge separatory funnel. The precipitated protein was successively re-extracted with 20 cc. and 15 cc. portions of butanol. The three butanol extracts were combined within the separatory funnel, vigorously extracted three times with 60 cc. of Blau's solution (4 N NaOH containing 5 per cent Na₂CO₂). Slow centrifugation (300 r.p.m.) for 5 minutes was used to break the emulsion which invariably formed. The alkalinized, washed butanol extract was transferred to a 150 cc. distilling flask and was distilled to dryness in vacuo at a temperature not exceeding 40°C. The dried residue was dissolved in 1.5 to 2.0 cc. of butanol or water and transferred to weighed planchets. Dilute HCl was added in order to reduce the alkalinity, and thereby to diminish the hygroscopic properties of the mixture. When the residue was taken up in water, an immiscible layer appeared, which contained almost all the bromphenol-blue and most of the thyroxine. Radioactivity in this phase was 3 to 8 times as great as in the aqueous phase. The chemical nature of this immiscible layer has not been determined. It does not originate from serum, since it can be demonstrated when saline, with or without added thyroxine, is extracted, and presumably, therefore, arises from either the apparatus or the reagents.

In the determination of the concentration of BEI^{IM} in erythrocytes, blood was drawn into a tube containing dried heparin. The erythrocytes were separated from the plasma and washed three times with normal saline solution. Five cc. of packed cells were laked with distilled water. Concomitant analyses for the concentration of BEI^{IM} in plasma and laked erythrocytes were then carried out as above.

The radioactivity of the samples was measured with an end-windowed (mica 1.4 to 1.8 mg. per cm.²) Geiger-Mueller tube and an automatic sample changer. At least 24,000 total counts were obtained for each serum sample. A correction, based on mass, was made in the observed radioactivity of each sample. Since, in the average euthyroid patient, the total thyroxine radioactivity in each sample of serum was approximately two-thirds of background radiation, approximately 9500 disintegrations were observed. The probable error of the determined radioactivity was therefore approximately 1 per cent. The accuracy of the counting procedure was considerably greater in hyperthyroid patients, in whom the concentration of radioactive thyroxine was usually markedly increased.

RESULTS

In an attempt to evaluate the analytic procedure described above, the recovery from serum of radio-active potassium iodide, diiodotyrosin,² and thyroxine ² was determined (Table I). From 88

TABLE I

Recovery of radioactive thyroxine, diiodotyrosine, and
potassium iodide during butanolextraction procedure

	Number	Per cent in	Per cent of added radioactivity in butanol extract*			
Radioactive substance added	of experi- ments	Prior to alkaline wash	alkaline alkaline			
		%	%	%		
Thyroxine	8	99.4	97.2	94.3		
Diiodotyrosine	4	47.1	0.05	0.02		
Potassium iodide	6	80.5	0.03	0.01		

^{*} Average values.

to 97 per cent of radioiodine added as thyroxine was recovered from the final residue, whereas I¹⁸¹ added as either potassium iodide or diiodotyrosine was virtually excluded.

In both butanol-acetic acid-water and collidine-ammonia systems (17), filter paper chromatography revealed that the radioactive material isolated from the serum of three patients who had received therapeutic doses of I¹⁸¹ moved precisely as did synthetic radiothyroxine. Both the material extracted from serum and synthetic radiothyroxine displayed small amounts of radioactivity with R.F.'s corresponding to those of iodide and diiodotyrosine. Degradation of thyroxine during the chromatographic procedure, leading to the appearance of similar fractions, has been previously described (17).

The recovery from serum of triiodothyronine was not determined. Furthermore the chromatographic systems employed would probably not have permitted resolution of this material from thyroxine. The data, therefore, offer no evidence concerning the extent, if any, to which radioactive triiodothyronine contributed to the observed values of the BEI¹⁸¹.

The concentration of BEI¹⁸¹ was determined in the washed erythrocytes of three patients who had received therapeutic doses of radioactive iodine.

TABLE II

Lack of transport of radioactive thyroxine in
human erythrocytes

Patient	BEIm	BEIm	
	(% dose/liter plasma)	(% dose/liter RBC)	
1	.301	.000	
2	.203	.000	
3	.214	.002	

² Obtained from Abbott Laboratories, Chicago, Illinois.

TABLE III—Summary of values obtained in diverse states of thyroidal function

	-		24-Hour BEI ¹²¹	72-Hour BEIss	24-Hour thyroidal I ¹³¹ uptake	24–72 Hour change in I ¹³¹ uptake*	BMR
Patient	Age	Sex	(% dose/liter)	(% dose/liter)	(% dose)	(% dose)	(% normal)
Euthyroidism							
1	59	F	.009	.062	41	– 0.8	– 5
2 3	37 35	F M	.012	.036 .03 5	33 37	- 0.4	16
4	42		.016 .01 4	.038	29	+ 1.1	-16 + 4
5	29	F F	.008	.023	25	+ 8.6	- 8
6	43	म	.011	.033	19	+ 4.9	-21
7	35	F F	.020	.063	28	- 1.9	20
8 9	38 27	F	.006 .014	.020 .043	30 40	+ 4.6 + 2.3; -2.5	-20 -10
10	26	F	.020	.047	39	+ 2.3, -2.3 + 2.1	- 10 - 2
11	29	F F	.022	.046	26	+2.0; -1.6	-17
12	58	F	.010	.030	34	- 1.3	-29
13 14	25 37	F F	.010	.028	29 35	- 0.2 + 2.2	+ 3
15	37 29	r M	.012 .008	.044 .022	17	+ 2.2 + 0.7	-20 + 8
16	5 9	M	.018	.040	36	+ 2.2; -1.9	+22
17	51	F	.018	.032	23	+ 0.5	+ 4
18	41	F	.032	.078	40	+ 1.0; -1.3	+ 3
19 20	37 27	F F	.017 .045	.051 .079	32 30	$\begin{array}{c} + 2.2 \\ + 3.6; -3.3 \end{array}$	$^{-26}$
20 21	47	F	.043 .019	.079	30 20	+ 3.0; -3.3 - 0.6	+ 2 + 6
22	41	F F F	.013	.032	32	$+\ 0.4;\ -0.2$, ,
23	28	<u>F</u>	.023	.028	20	+ 2.3; -1.5	- 5
24	55 40	F	.018	040	20	+ 1.9	- 5
25 26	38	F F F	.028 .020	.050 .057	21 43	+ 0.1 - 5.4	- 3 -28
27	44	M	.010	.038	24	+ 1.9: -2.2	-33
28	52	<u>M</u>	.012	.017	29	+ 1.4	-10
29	44	F	.005	.041	28	+ 0.8; -1.0	
30 31	33 32	F	.010 .014	.033 .054	31 30	+4.0; -2.0 -1.0	$^{+ 6}_{-11}$
32	43	F F	.030	.053	32	- 0.6	-18
33	58	M	.004	.015	37	+ 7.8	-30
34	27	F F	.014	.043	37	+ 3.2; $-$ 1.3	+ 7
35 36	57 32	r F	.005 .016	.016 .015	32 32	$+\ 3.4;\ -1.5 \\ +\ 2.0$	- 5
37	35	F	.010	.053	44	+ 0.5; -0.7	+15
38	31	F F	.022	.036	39	+ 2.0; -0.7	+19
39	49	F F	.008	.020	14	+ 0.5; -0.2	- 4
40 41	50 33	F	.041 .025	.067 .067	49 32	$\begin{array}{c} + 0.6; -0.3 \\ - 0.5 \end{array}$	-30 -13
42	55	F	.012	.051	39	- 0.3 + 0.7	13
43	27	F F	.013	.020	57	+ 1.8	- 4
44	30	<u>F</u>	.008	.030	29	+ 0.9	-14
45	71	F F	.018	.021	18	+ 1.9	- 6
46 47	48 30	Р М	.014 .008	.021 .019	3 4 18	$\begin{array}{c} + 2.5; -1.5 \\ + 0.2; -1.2 \end{array}$	-24
48	33	M	.022	.042	25	+ 2.0	-23
49	48	M	.010	.062	26	+6.0; -4.0	-21
50	36	F	.007	.018	13	- 1.2	- 6
51 52 53	15 38	7 T	.033 .017	.044 .035	24 34 55	+ 2.3 - 5.4	- 9 - 20
53	31	F	.014	.020	55		+ 2
54	62	F	.010	.024	27	- 0.2	. 8
55 56	33	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	.012	.038	26	+ 0.6	-29 + 2 - 8 + 4 + 4
50 57	38	F	.010 .012	.022 .014	17 40	+ 3.4	+ 4
57 58	26 32	r F	.012 .016	.035	40 23	- 0.8	0
58 59	28	F	.018	.018	16		
60	28	M	.020	.039	18 44		+ 5 +13
61 62	62 33 38 28 32 28 28 32 32 35	F	.014	.041	44 26	+ 5.3; -2.0	+13
62 63	35 30	M M	.009 .015	.024 .052	26 25		-10
64	28	M	.012	.032	25 21		-12
		/lean	.016	.037	30	+ 0.1	•
		td. Dev.	.008	.016	9	2.6	

^{*} Two figures given in patients demonstrating Pattern No. 2 (see Text). The negative value in these cases is used in calculations.

TABLE III-Continued

Patient	Age	Sex	24-Hour BEI ¹²¹ (% dose/liter)	72-Hour BEI ¹³¹ (% dose/liter)	24-Hour thyroidal I ¹³¹ uptake (% dose)	24-72 Hour change in I ¹³¹ uptake* (% dose)	BMR (% normal)
			H	yperthyroidism			
65 66	37 22	F M	.747 .018	1.085 .023	80 65	-18.5 - 1.2	+50 +37
67 68	46 29	F M	.151 .036	.338 .204	94 76	-22.1 - 6.7	+41 +35
69	22	F	.568	.712	96	- 5.6	+50
70 71	28 44	F F	.019 .053	.250 .127	38 77	- 3.2 - 4.9	+37 +42
72	42	F	.033	.150	97	- 4.9 - 4.4	+55
73	60	M	.141	.308	66	+9.3; -4.1	
74 75	53 58	F F	.198 .202	.468 .549	65 75	$\begin{array}{c} + 2.0; -2.7 \\ -25.8 \end{array}$	+28 +37
76 76	29	M	.034	.253	57	- 23.8 - 4.7	+ 49
77	28	M	.104	.283	95	- 9.7	+40
78 79	51 30	F F	.230 .104	.548 .429	69 48	-16.3 +10.4; -2.0	+72 +30
80	41	F	.081	.327	56	+ 0.2: -2.0	+33
81	37	F F F	.054	.163	55	+6.0; -2.5	+30
82 83	35 27	F	.850 .488	1.170 1.040	76 94	- 6.0	+62
84	37	F	.340	.862	76	-12.3	+55
85	28	F	.019	.079	42	- 0.4	+18
86 87	23 41	M F	.047 .166	.080 .301	56 80	+ 3.3 - 3.2	+30 +42
88	59	F	.020	.081	45	5.2	+25
89	62	F	.359	.611	82		+38
		lean td. Dev.	.205 .232	.418 .104	70 18	- 7.0 7.2	
			Thy	roidal Inactivi	ty		
90†	32	F	.002	.002	2		-38
91‡ 92‡	23 24	M M	.003 .004	.004 .006	19 17		-28 -30
93 †	28	F	.004	.003	10		-35
94†	14	F	.006	.008	2		-30
95§ 96§	35 36	F M	.002 .010	.006 .011	1 7		+15
97 1	45	F	.003	.002	1		-38
98†	50	F	.004	.004	2 2 4		-40
99† 100‡	28 47	F M	.006 .006	.007 .006	4		-36 -25
101‡	55	F	.003	.008	4		$-\frac{20}{27}$
102†	30 36	F	.006	.005	3		-45
103§	36	M	.004	.008	-		
		lean td. Dev.	.004 .002	.006 .003	6 6		
			Post	-Thyroidectom	у		
104	24 8 8	F F F	.318	.066	7		-38 -30
105 105a¶	8 9	F	.22 4 .162	.159 .040	7 4 3 4 2 2 9		-30
106	29	M	.121	.078	3		-35
106a	29 18	M	.090 .088	.083	4		
107 108	18 28	F M	.088 .010	.025 .037	2		-37 -29
108a ·	28	M	.060	.048	õ		
109	4 0	F	.072	.080	15		-25
109a	40	F	.069	.086	21		
	N S	Mean Std. Dev.	.121 .091	.070 .038	7 6		

[†] Primary myxedema.
‡ Hypopituitarism.
\$ Euthyroid receiving exogenous thyroid.
¶ All patients in this group underwent thyroidectomy for thyroidal carcinoma.
¶ Repeat test in same patient.

Although simultaneously determined concentrations of BEI¹³¹ in the plasma were high, the radioactivity extracted from the erythrocytes was indistinguishable from background radiation (Table II).

In all euthyroid and hyperthyroid patients, the concentration of BEI181 in the serum increased during the interval between 24 and 72 hours after the administration of the tracer (Table III). Patients with decreased thyroidal function associated with primary or pituitary myxedema, or with the ingestion of thyroid substance shall hereafter be referred to as patients with thyroidal inactivity. In this group of cases, significant differences between the 24 and 72 hour concentrations could not be detected. In five of six patients with carcinoma of the thyroid in whom total thyroidectomy had been attempted, and in two patients with thyrotoxicosis who continued to take propylthiouracil throughout the period of observation (not shown in Table III) the 24-hour concentration of BEI¹⁸¹ exceeded that found at 72 hours.

The mean values of the BEI¹⁸¹ concentrations in patients with thyrotoxicosis, euthyroidism, or thyroidal inactivity differed significantly from that found in the other two groups (p < .01), both at the 24-hour and at the 72-hour period. However, a better separation between the values found in patients with thyroidal inactivity, euthyroidism, and hyperthyroidism was found at the 72-hour period (Figure 1). In only one patient presumed to have thyrotoxicosis did the 72-hour concentration of BEI181 fall definitely within the normal range. This patient (No. 66) was a 22-year old male with severe exophthalmos, goiter, a thyroidal radioiodine uptake of 65 per cent at 24 hours, and very mild symptoms of thyrotoxicosis. In three patients in whom the diagnosis of thyrotoxicosis had been made, the BEI181 values were just above the range of normal. Of these patients one (No. 86) was a young male with exophthalmos, goiter, a 56 per cent radioiodine uptake, and minimal symptoms of thyrotoxicosis. The other two patients (No. 85, No. 88) were anxious women in whom the diagnosis of thyrotoxicosis was in doubt at the outset.

In one patient (No. 96) with thyroidal inactivity, the BEI¹⁸¹ value fell at the lower end of the normal range. This patient was a 36-year old male with non-toxic nodular goiter, who was re-

ceiving 2 gr. of dessicated thyroid substance daily, and whose 24-hour radioiodine uptake was 7 per cent.

In hyperthyroid patients, the mean net change in thyroidal uptake between 24 and 72-hours (-7.0 per cent) was significantly different (p < .01) from that found in euthyroid subjects (+0.1 per cent). This was also true when the net change was calculated in each patient as percentage of the 24-hour radioactive iodine uptake. (-9.0 per cent vs. + 0.6 per cent). Successive determinations of thyroidal uptake at 24, 48, and 72 hours disclosed three patterns. The first was a progressive increase in the thyroidal content of radioiodine, the second an increase followed by a decrease, and the third a successive decrease. Of the euthyroid patients, 41 per cent demonstrated pattern 1, 36 per cent, pattern 2, and 23 per cent pattern 3. Of the hyperthyroid patients, 4.6 per cent (one patient) demonstrated pattern 1, 18.2 per cent pattern 2, and 77.2 per cent pattern 3. The severity of thyrotoxicosis could not be consistently correlated with the pattern of consecutive neck counts. However, those patients with the most severe thyrotoxicosis generally demonstrated pattern 3, while those with the mildest disease displayed either pattern 1 or a small net loss of radioactivity of the type found in pattern 2.

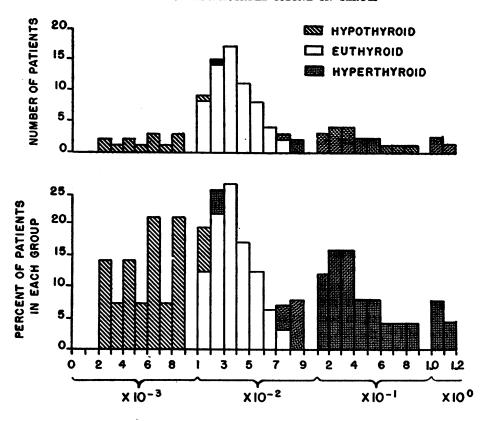
In patients with decreased thyroidal function, the low level of radioactivity in the neck precluded the accurate measurement of progressive changes

TABLE IV

Correlation between the functions studied in various clinical groups

Functions	r	p
24 Hr. BEI ¹⁸¹ vs. 72 Hr. BEI ¹⁸¹		
Euthyroid	0.59	<.001
Hyperthyroid	0.91	<.001
Combined*	0.63	<.001
72 Hr. BEI181 vs. 24 Hr. thyroidal u	ptake	
Euthyroid	0.23	>.05
Hyperthyroid	0.35	>.05
Combined	0.66	<.001
72 Hr. BEI131 vs. "Net change"†		
Euthyroid	-0.34	<.01
Hyperthyroid	-0.44	<.02
Combined	-0.40	<.001
24 Hr. thyroidal uptake vs. "Net cl	nange"	
Euthyroid	-0.06	>.05
Hyperthyroid	-0.20	>.05
Combined	-0.61	<.001

^{*}Euthyroid and hyperthyroid groups considered together.
† 24–72 Hour change in thyroidal I¹⁸¹ uptake.



SERUM CONCENTRATION OF BE 1131- PERCENT ADMINISTERED DOSE/LTR.

FIG. 1. DISTRIBUTION OF 72-HOUR CONCENTRATIONS OF BUTANOL-EXTRACTABLE I^{IM} IN THE SERUM OF HYPERTHYROID, EUTHYROID, AND HYPOTHYROID PATIENTS (EXCLUDING PATIENTS WITH THYROIDAL CARCINOMA)

in the uptake of radioactive iodine. Nevertheless, it appeared certain that some thyroidal accumulation and storage of I¹⁸¹ had occurred, since measureable radioactivity persisted in the region of the thyroid gland for several days.

Statistical correlations between the various functions studied are presented in Table IV.

DISCUSSION

Recovery experiments have demonstrated that the extraction procedure described is relatively specific for the principal iodine-containing compound in the plasma, thyroxine. In confirmation of previous reports, thyroxine has been found to be completely absent from the erythrocyte (18).

Measurement of the concentration of radioactive thyroxine in the serum following the administration of a standard dose of radioiodine has been found to be a rather reliable guide to the state of thyroidal function in patients with thyrotoxicosis, euthyroidism, and thyroidal inactivity. In several patients who had undergone virtually complete thyroidectomy, however, the concentration of BEI¹⁸¹ did not correlate well with the clinical status of the patient. An explanation for this discrepancy was sought in an analysis of the factors determining the concentration of BEI¹⁸¹ in the plasma.

The principal and perhaps only source of plasma's thyroxine appears to be the pool of hormonal iodine within the thyroid gland. Therefore, the relationship described by Zilversmit, Entenman, and Fishler for a product substance and its immediate precursor can be applied to the relationship existing between thyroxine in the plasma and in the thyroid gland (19). These authors have demonstrated that when the precursor, and thence the product, is labelled with a radioactive component, the specific radioactivity (radioactive substance

X/total substance X) of the precursor initially exceeds that of the product. The specific activity (S.A.) of the product then rises with a rapidity which is, at any time, proportional to the magnitude of the difference between the S.A.'s of the precursor and the product. The peak S. A. of the product is reached when the specific activities of the two have become equal. As the S.A. of the product exceeds that of the precursor, its own S.A. begins to decline. If it be assumed that the content of non-radioactive hormonal iodine in the thyroid gland and in the plasma remain constant during the period of observation, then changes in the radioactivity of the thyroid gland or of the plasma's BEI will be proportional to changes in their respective S.A.'s.

The concentration of hormonal iodine in the plasma of hyperthyroid patients is greater than normal (20, 21). Nevertheless, in such patients following a tracer dose of I¹⁸¹, the S.A. of the PBI increases more rapidly than normal (22). The present findings suggest that the rate of increase of the S.A. of the BEI is also augmented in patients with thyrotoxicosis. In such patients, the S.A. of the glandular hormonal iodine must therefore exceed, at least initially, that found in patients with normal thyroidal function. The relatively greater S.A. of the thyrotoxic gland commonly results from an increase in the initial accumulation of I¹⁸¹ and from a reduction in the total quantity of hormone stored within the gland (23).

The "net change" in glandular radioactivity describes the direction and magnitude of changes in the S.A. of organically-bound iodine within the gland during the 24 to 72-hour interval. A decrease in the S.A. of the glandular hormone * was seen more often in thyrotoxic than in normal patients. The average thyroidal S.A. increased by 0.6 per cent in normal patients, but it decreased by 9 per cent in thyrotoxic patients. The more rapid loss of radioactivity from the thyroid glands of thyrotoxic patients results from an increase in the rapidity of disappearance of radioiodide from the plasma (24) which hastens the cessation of collection of radioiodine, and from an increase in the rate of turnover of glandular hormone, as will be seen below.

Thus in thyrotoxic patients, the S.A. of the

plasma's hormone is increasing and that of the glandular hormone is decreasing more rapidly than normal. The time interval required for the two to become equal and for the concentration of BEI¹⁸¹ to reach a peak, should therefore be shorter than normal in thyrotoxic patients. This proves to be the case when re-utilization of radioiodide made available by the peripheral degradation of hormone is prevented (22). However, when as in the present study, re-utilization of radioiodide is allowed to occur, the decline in the S.A. of the glandular hormone is retarded, the peak concentration of the plasma's BEI¹⁸¹ is delayed, and its level increased.

In the patients "totally" thyroidectomized for thyroidal carcinoma, the concentration of BEI181 in the serum decreased during the interval between 24 and 72 hours after the administration of the tracer. The S.A. of the BEI in the plasma of these patients must, therefore, have exceeded that of the glandular hormone more quickly than normal. This could be explained by either a more rapid decrease in the S.A. of the glandular hormone, a more rapid increase in the S.A. of the plasma's hormone, or both. All patients who demonstrated this pattern were hypothyroid, and in three the PBI was found to be subnormal. Since these patients demonstrated a normal or increased concentration of BEI¹⁸¹ in their serum at 24 hours, it may be concluded that the S.A. of their circulating hormonal iodine had increased more rapidly than normal. However, the presence in the plasma of normal or increased concentrations of BEI181 suggests that rather large amounts of radioactive hormone had been released. Furthermore, the hypothyroid state of these patients indicates that the total amount of hormone released by their thyroid tissue per unit time was subnormal. Thus the S.A. of the hormone released by these glands must have been greater than normal. This conclusion, together with the markedly decreased initial uptake of I181 found in the thyroid glands of these patients, indicates that the radioactive hormone formed was minimally "diluted" by preformed, non-radioactive hormone. The total amount of hormone stored by these thyroid glands must, therefore, have been considerably decreased.

A similar decrease in the concentration of BEI¹⁸¹ between 24 and 72 hours after the administration of the dose was noted in the serum of two pa-

⁸ The term "hormone," used in this sense, refers to all iodinated tyrosyl compounds within the gland.

tients with thyrotoxicosis made euthyroid by the administration of propylthiouracil. This agent reduces the rate of formation of thyroid hormone (25, 26) but does not diminish, and may rather increase the rate of release of hormone from the gland (27). In the hyperthyroid patient, this must result in a marked depletion of glandular hormone.

It is apparent that the concentration of BEI¹⁸¹ in the serum does not always accurately reflect the overall rate of hormone formation or release. For any given rate of radioiodine accumulation, depletion of glandular hormone results in an increased release of BEI¹⁸¹. To the extent that depletion of glandular hormone occurs in patients with thyrotoxicosis, the sensitivity of the test in detecting the hyperthyroid state is increased. However, when depletion of glandular hormone occurs in patients who are not thyrotoxic, the concentration of BEI¹⁸¹ in the serum does not correlate well with the clinical state. Two cases of postoperative myxedema demonstrating this phenomenon have recently been reported (28).

It is possible to evaluate further the manner in which the amount of glandular hormone influences the concentration of BEI¹³¹ in the serum. It will be assumed that the maximal accumulation of radioiodine within the thyroid gland is instantaneous, and that within the first three days after the administration of the tracer, peripheral utilization of released radioactive hormone is negligible.⁴

If A = thyroidal content of I¹⁸¹ at time, t (per cent of administered dose).

A₀ = the peak thyroidal accumulation of I¹⁸¹ (per cent of administered dose).

(BEI¹⁸¹) = concentration of BEI¹⁸¹ in the serum at time, t (per cent dose/liter serum).

V = volume-of distribution of BEI¹⁸¹ (total extrathyroidal BEI¹⁸¹/(BEI¹⁸¹).

- r = rate of turnover of glandular hormone (per cent of glandular hormone synthesized or released per day).
 - t = time after peak thyroidal accumulation of I¹⁸¹ (days).

The amount of I¹⁸¹ within the thyroid gland at any time,

$$A = A_0 e^{-rt}$$

The rate of release of radioactive hormone,

$$\frac{d(BEI^{181})V}{dt} = rA_0e^{-rt}$$

Therefore, the total amount of BEI¹⁸¹ released up to time, t,

$$(BEI^{131})V = -A_0e^{-rt} + C$$

Since when t = 0, (BEI¹⁸¹) V = 0,

 $C = A_0$

and

$$(BEI^{131})V = A_0(1 - e^{-rt})$$
 (1)

At any time, and for any value of V and A₀, the concentration of BEI¹⁸¹ in the serum will depend upon the rate of turnover of glandular hormone (r). Therefore, at any given rate of hormone manufacture, decreases in the quantity of glandular hormone increase the concentration of BEI¹⁸¹ in the serum by increasing the rate of turnover of hormone within the thyroid gland.

Unlike the thyroid tissue of patients designated as having thyroidal inactivity, that of the patients who had been thyroidectomized is characterized by a high rate of turnover of glandular hormone. It is not known whether, in these patients the remaining thyroid tissue is normal or neoplastic in character. If it were the latter, then the increased turnover rate could be the result of an inability of the tissue to store iodine because of a structural characteristic, such as absence of follicles (29). It is more likely, however, that the increased turnover rates reflect an intense stimulation of the residual thyroid tissue by thyrotropin (30). In the latter instance, the failure of the residual tissue to maintain a euthyroid state could result solely from an inadequate glandular mass. In either case, attempts to alter the function of these thyroidal remnants in such a manner as to make possible therapy with radioactive iodine might well be directed towards increasing the thyroidal storage

⁴ It is, of course, recognized that the maximal thyroidal accumulation of I^m is not instantaneous. Furthermore, it is likely that considerable utilization of radioactive hormone occurs during the 72-hour period of observation, especially in patients with thyrotoxicosis. Nevertheless, the mathematical relationships defined are considered to be first approximations to those which truly pertain to the functions under consideration.

of hormone as well as increasing the avidity for iodine.

Equation 1 suggests that it may be possible to evaluate the rate of turnover of glandular hormone by examining the relationship between the BEI¹⁸¹ and the initial accumulation of radioiodine within the thyroid gland. From equation 1, the slope of the curve representing the relationship between these two functions may be described as

$$\frac{d(BEI^{181})}{dA_0} = \frac{(1 - e^{-rt})}{V}$$
 (2)

For any value of t, the slope will be a straight line, provided that both V and r are constant.

Figure 2 illustrates the relationship observed in the present study between the concentration of BEI¹⁸¹ in the serum 72 hours after the administration of the tracer and the 24-hour thyroidal uptake. The values displayed by patients with thyroidal carcinoma, although shown on the chart, are not included in the calculations made from this figure. In each of the other patients, the observed 24-hour thyroidal uptake of radioiodine is "corrected" for the amount of radioactive hormone which has been released by this time. This correction is made by adding to the observed uptake the product of the 24-hour concentration of BEI181 in the serum and an assumed volume of distribution of BEI¹⁸¹ of 24 liters (27). The curve shown is the visual best fit to a series of points representing the average value of all patients within each 10 per cent uptake-interval. Rather than following a straight line, the slope of the curve rises sharply as the uptake approaches 100 per cent. Since the volume of distribution of thyroid hormone apparently does not differ among patients with varying states of thyroidal function (27), equation 2 indicates that as the maximal uptake increases, the rate of turnover of glandular hormone increases. This conclusion is in accord with direct measurements, which reveal an increased rate of release of hormone from thyrotoxic glands (31).

The curve shown in Figure 2 is described by the equation

Uptake =
$$\frac{100(BEI^{131})}{.09 + (BEI^{131})}$$
 (3)

However, the relationship between the thyroidal rate of clearance of plasma iodide and the uptake of radioactive iodine has been described previously by the following equation (32):

Uptake =
$$\frac{100 \times \text{thyroidal iodide clearance } (C_t)}{\text{thyroidal clearance } (C_t)}$$

+ renal clearance (C_r)

Therefore, from equations 3 and 4,

Uptake =
$$\frac{100(BEI^{121})}{.09 + (BEI^{121})} = \frac{100C_t}{C_t + C_r}$$
 (5)

(BEI¹⁸¹) =
$$\frac{.09 \, C_t}{C_r}$$
 (6)

If the average normal rate of renal clearance of iodide is assumed to be 2.0 liters per hour (33),

$$(BEI^{181}) = .045 C_t$$
 (7)

where C_t = thyroidal iodide clearance rate (liters per hour).

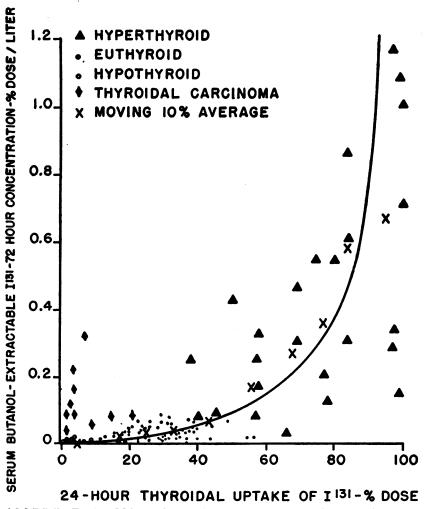
The BEI¹⁸¹ concentration in the serum of patients with hyperthyroidism, euthyroidism, and thyroidal inactivity, therefore, appears to be directly proportional to the thyroidal rate of clearance of plasma radioiodide. This conclusion is in accord with the a priori assumption that the thyroidal rate of accumulation of iodide is equal to the rate of release of hormonal iodine. Employing equation 7, and the average normal value for the BEI181 found in the present study, a thyroidal clearance rate of 0.78 liters per hour may be derived, which is in accord with direct measurements of this function (33-35). From equation 1, together with equations 3, 4, and 7, it is possible to estimate the average rate of turnover of thyroidal hormone for any value of the thyroidal clearance rate, the 72hour BEI¹⁸¹, or thyroidal uptake of radioactive iodine. It can be shown (see Appendix) that

$$-r = \frac{\ln (.9784 - .0108 C_t)}{3}$$
 (8)

$$-r = \frac{\ln (.9784 - .24(BEI^{181}))}{3}$$
 (9)

$$-r = \frac{\ln\left(1 - \frac{2.16}{100 - A_0}\right)}{3} \tag{10}$$

From equation 9, the turnover rate associated with the average normal value of the BEI¹⁸¹ found in the present study is approximately 1 per cent per day. There is considerable evidence that the aver-



(CORRECTED FOR DISCHARGED RADIOACTIVE HORMONE)

Fig. 2. The Relationship between 24-hour Thyroidal Uptakes of I¹⁸¹ and 72-hour Concentrations of Butanol-Extractable I¹⁸¹ in Serum

Thyroidal uptakes have been "corrected" by adding to the observed uptake the quantity of radioactive hormone released during this interval (24-hour BEI³⁵ × assumed volume of distribution of 24 liters). The curve shown represents the visual best fit to a series of moving averages of 10 per cent-uptake intervals. Values obtained in patients with thyroidal carcinoma have been excluded from calculations of interval-averages.

age normal daily production of thyroxine is approximately 70 mcg. daily (27). The hormone content of the average normal thyroid gland can, therefore, be estimated to be approximately 7000 mcg., which value agrees closely with those obtained by direct chemical analysis (36, 37).

SUM MARY

1. A method for the determination of the BEI¹⁸¹ of serum has been shown to measure principally the

radioactivity of circulating thyroxine, and has been applied to the study of the concentration of BEI¹⁸¹ in the serum of 109 patients with various states of thyroidal function.

- 2. These results have been correlated with the 24-hour thyroidal uptake of radioiodine and with the subsequent change in thyroidal radioiodine content during the 24-72 hour period.
- 3. Analysis of the results reveals that the concentration of BEI¹⁸¹ in the serum is dependent on

the initial thyroidal uptake of I¹⁸¹, the rate of synthesis of thyroid hormone, and the amount of hormone stored within the gland.

4. The 72-hour concentration of BEI¹⁸¹ correlates well with the clinical state except in non-thyrotoxic patients in whom the quantity of hormone stored within the gland is markedly decreased.

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APPENDIX

From equation 1 (see Text),

$$(BEI^{131})V = A_0(1 - e^{-rt})$$

substituting for (BEI 181) and A_0 from equations 4 and 7

.045
$$C_t V = \frac{100 C_t}{C_t + C_r} (1 - e^{-rt})$$

where V = 24 liters, $C_r = 2$ liters per hour, and t = 3 days,

$$\begin{split} 1.08 \, C_t &= \frac{100 \, C_t}{C_t + 2} \, (1 - e^{-3r}) \\ 1.08 \, C_t{}^2 + 2.16 \, C_t &= 100 \, C_t (1 - e^{-3r}) \\ .0108 \, C_t + .0216 &= 1 - e^{-3r} \\ e^{-3r} &= .9784 - .0108 \, C_t \\ -r &= \frac{\ln \, (.9784 - .0108 \, C_t)}{3} \end{split}$$

Furthermore, from equation 7,

$$C_t = \frac{(BEI^{131})}{.045}$$

Therefore,

$$-r = \frac{\ln .9784 - .24 \text{ (BEI^{181})}}{3}$$

According to equation 3,

$$A_0 = \frac{100(BEI^{131})}{.09 + (BEI^{131})}$$

$$.09 A_0 + A_0(BEI^{131}) = 100(BEI^{131})$$

$$(BEI^{131}) = \frac{.09 A_0}{100 - A_0}$$

Therefore, according to equation 1,

$$\begin{split} \frac{.09 \, A_0 V}{100 \, - \, A_0} &= A_0 (1 \, - \, e^{-3r}) \\ \frac{.09 \, \times \, 24}{100 \, - \, A_0} &= 1 \, - \, e^{-3r} \\ \frac{2.16}{100 \, - \, A_0} &= 1 \, - \, e^{-3r} \\ e^{-3r} &= 1 \, - \, \frac{2.16}{100 \, - \, A_0} \\ -r &= \frac{\ln \left(1 \, - \, \frac{2.16}{100 \, - \, A_0} \right)}{3} \end{split}$$

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